Different Transcriptional Activity and *In Vitro* TNF-α Production in Psoriasis Patients Carrying the TNF-α 238A Promoter Polymorphism

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Genes encoded on chromosome 6 within the major histocompatibility complex region are thought to play an important role in the pathogenesis of psoriasis. A potential candidate gene is tumor necrosis factor α. The tumor necrosis factor α promoter contains several polymorphisms including two G→A transitions at position –308 and –238, which are the most common in Caucasian populations. The TNF238.2 (–238A) allele has been strongly associated with psoriasis. We have investigated the effect of the –238 and –308 variants on transcription of the tumor necrosis factor α gene in luciferase reporter gene assays. In addition, peripheral blood mononuclear cells of 47 patients with psoriasis and 43 controls were stimulated with different antigens and mitogens (streptococcal sonicate and superantigen, lipopolysaccharide, phorbol-12-myristate, phytohemagglutinin, CD3 antibodies) and tumor necrosis factor α production was measured in supernatants by enzyme-linked immunosorbent assay. The psoriasis-associated tumor necrosis factor α promoter allele TNF238.2 showed a significantly decreased transcriptional activity. Peripheral blood mononuclear cells carrying this allele produced significantly less tumor necrosis factor α after stimulation with T cell mitogens and streptococcal antigens in comparison to controls. The promoter allele TNF238.2 seems to influence tumor necrosis factor α production; a possible role in the pathogenesis of psoriasis has to be further evaluated. ***Key words: PBMG/promoter polymorphism/psoriasis/TNFα/transcriptional activity. J Invest Dermatol 114:1180–1183, 2000***

Psoriasis is a polygenic, T-cell-mediated skin disease with a heterogeneous genetic background (Bos and de Rie, 1999). One of the most consistent associations exists with genes of the major histocompatibility complex (MHC) on the short arm of chromosome 6 (Elder *et al*, 1994). Although recent studies have located a potential candidate gene between human leukocyte antigen B (HLA-B) and HLA-C (Allen *et al*, 1999), the considerable genetic heterogeneity of psoriasis suggests that other nearby encoded genes could act as disease modifiers.

Tumor necrosis factor α (TNF-α) is encoded centromerically to HLA-B within the class III region. TNF-α is an important inflammatory mediator that is enriched in early psoriatic lesions (Ettehadi *et al*, 1994). Several polymorphisms in the TNF-α region have been described, including five microsatellites and several point mutations particularly in the promoter region (Jongeneel *et al*, 1991; Nedospasov *et al*, 1991; Skoog *et al*, 1999). In Caucasian populations the most common exchanges are two G→A transitions in the promoter at positions –308 (308A/TNF308.2) and –238 (238A/TNF238.2) (Wilson *et al*, 1992; D'Alfonso and Richiardi, 1994). A rare polymorphism at promoter position –376 occurs strictly linked to the TNF238.2 allele (Hamann *et al*, 1995; Knight *et al*, 1999). We and colleagues have found a strong association of the polymorphism at position –238 with psoriasis and psoriatic arthritis (Arias *et al*, 1997; Hoehler *et al*, 1997).

The functional consequences for the TNF238.2 allele are not yet clear (Pociot *et al*, 1995). Contradictory findings exist for the TNF308 polymorphism (Stuber *et al*, 1996; Kroeger *et al*, 1997; Wilson *et al*, 1997). In this study we have investigated the effect of the –238 polymorphism on transcriptional activity of the TNF-α promoter in comparison with another polymorphism at position –308. In addition we have studied the influence of these TNF-α promoter polymorphisms on TNF-α production of peripheral blood mononuclear cells (PBMC) in psoriasis patients after stimulation with different mitogens and streptococcal antigens.

PATIENTS AND METHODS

Patients Forty-seven patients with type I psoriasis (15 with psoriatic arthritis) from the Department of Dermatology and the First Medical Department (Rheumatology), University Clinic of Mainz, Germany, were recruited for this study. Psoriatic arthritis was defined as a seronegative inflammatory arthritis, associated with psoriasis (Gladman, 1994). All patients were seen by a rheumatologist (EMH). Thirty-nine male and eight female patients were included; mean age was 48.8 y (range 20–83 y) and 59.1 y (range 46–82 y), respectively. Female patients were only included after menopause. At the time of recruitment, none of the patients was receiving immunosuppressive treatment like corticosteroids, methotrexate, cyclosporine A or ultraviolet A therapy. Patients did not suffer from any other relevant disease nor did they show any signs of streptococcal infections at the time of recruitment. The healthy control population consisted of 43 Caucasoid male persons, mean age 38.3 y (range 23–91 y).

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Abbreviation: PBMC, peripheral blood mononuclear cells.

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Detection of TNF-α promoter polymorphisms The polymorphisms at positions –238 and –308 were studied as previously described (Wilson *et al*, 1992; Gallagher *et al*, 1997). In addition samples carrying the –238A exchange were sequenced for the detection of the –376 polymorphism.

TNF-α enzyme-linked immunosorbent assay PBMC were isolated from freshly taken heparinized blood by standard Ficoll-Hypaque gradient centrifugation. All experiments were performed in duplicates in 96 well microtiter plates (Nunc, Roskilde, Denmark) with 10^5 mononuclear cells in 100 µl assay medium per well. The following mitogens and antigens were added: 10 ng per ml lipopolysaccharide (LPS; Sigma, Deisenhofen, Germany), 10 ng per ml phorbol-12-myristate (PMA; Sigma) (both stimulators of B cell and monocyte/macrophage proliferation); T cell mitogens - 1 µg per ml anti-CD3 monoclonal antibodies (CD3), 1 µg per ml phytohemagglutinin (Difco Labs, Detroit, MI), 0.1 µg per ml *Streptococcus pyogenes* sonicate, and 0.25 µg per ml streptococcal superantigen SPEC. Cells were incubated for 24 h at 37°C and 5% CO_2 in a humified atmosphere. TNF-α production was measured in supernatants using a PharminGen TNF-α test kit (Pharmin-Gen, Hamburg, Germany).

Cell lines and culture conditions Human T cell derived Jurkat and B-lymphoblastoid Raji cell lines were maintained in culture medium consisting of RPMI-1640 supplemented with 2 mM L-glutamine, 100 U per ml of penicillin, 100 µg per ml of streptomycin and 5% fetal bovine serum, at 37°C with 5% CO_2.

Construction of luciferase fusion plasmids The three naturally occurring allelic forms of the TNF-α promoter (wild-type, TNF308G/238G; TNF308.2, TNF308A/238G; TNF238.2, TNF308G/238A) were cloned into the luciferase reporter gene construct (**Fig 1**). A TNF308A/238A allele has never been reported as mutations have occurred independently on different haplotypes. A 691 bp fragment (position –585 to +106) of the TNF-α gene was amplified by polymerase chain reaction (Wilson *et al*, 1997), cloned into the *pGL*3.basic-vector (Promega, Madison, WI), digested with *Xho*I (New England Biolabs, Schwalbach, Germany) and *Hind*III (New England Biolabs), and used to transform *Escherichia coli* (strain INVαF′, Invitrogen, Groningen, The Netherlands). Prior to transfection experiments plasmids were sequenced by the dideoxy chain terminator method and analyzed on an ABI 310 DNA sequencer (Applied Biosystems, Weiterstadt, Germany).

Transient transfections and luciferase assays Jurkat and Raji cells were transfected during their log growth phase by DEAE transfection with plasmid DNA prepared and purified using Qiagen Maxi prep-250 Kit (Qiagen, Hilden, Germany). A total of 10^7 cells were transfected with 10 µg of construct DNA plus 2.5 µg pcDNA3.1/HisB/*lacZ* control plasmid (Invitrogen). After transfection cells were distributed into two wells and incubated for 16 h (Jurkat) or 26 h (Raji). Cells were either left unstimulated or were induced for 8 h (Jurkat) or 15 h (Raji) with Ionomycin (1 µg per ml) and PMA (50 ng per ml) to achieve maximal stimulation.

Luciferase and β-galactosidase activity of cell lysates was measured according to the manufacturers' instructions (Promega, Mannheim, Germany; Tropix, Bedford, OH). All transfections were performed five to six times. Luciferase activity was normalized against β-galactosidase activity.

Statistical analysis Wilcoxon and Kruskal-Wallis tests were used for statisitical analysis of enzyme-linked immunosorbent assay data. The results of the luciferase assays were analyzed by variance analysis.

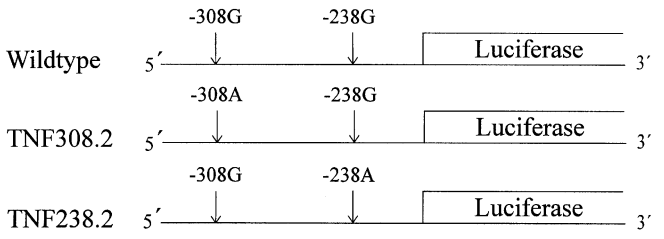

Figure 1. Schematic presentation of the reporter gene constructs used for transfection assays.

Table I. TNF-α promoter genotype distribution in groups of patients

Genotype		Patients (n = 47)		Controls (n = 43)	
–308	–238	n	%	n	%
G/G	G/G	28	59.5	28	56.1
A/G	G/G	5	10.6	10	23.2
G/G	A/G	14[a]	29.8	5	11.6

[a]p < 0.02.

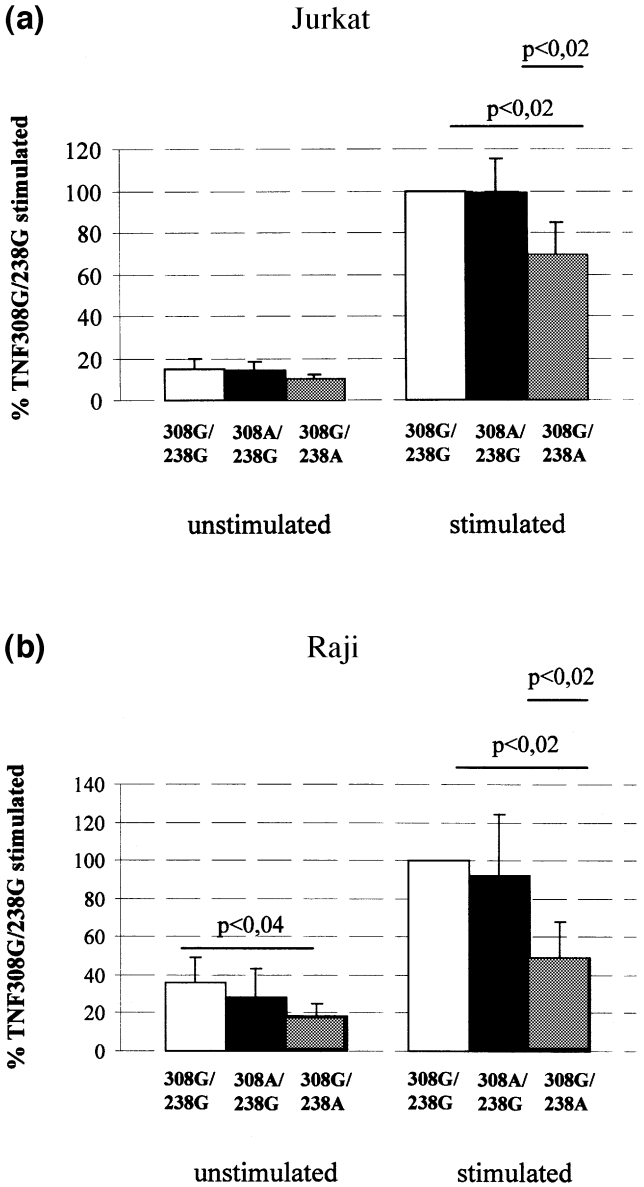

Figure 2. Results of the luciferase reporter gene assays comparing the different promoter variants. Activity of the wild-type reporter gene construct (308G/238G) in stimulated cells was set at 100%; the constructs TNF308.2 (308A/238G) and TNF238.2 (308G/238A) are expressed in relation to this. Cells were either left unstimulated or were induced with PMA and Ionomycin. The *bars* represent means of six independent transfections. (*a*) Jurkat cells; (*b*) Raji cells.

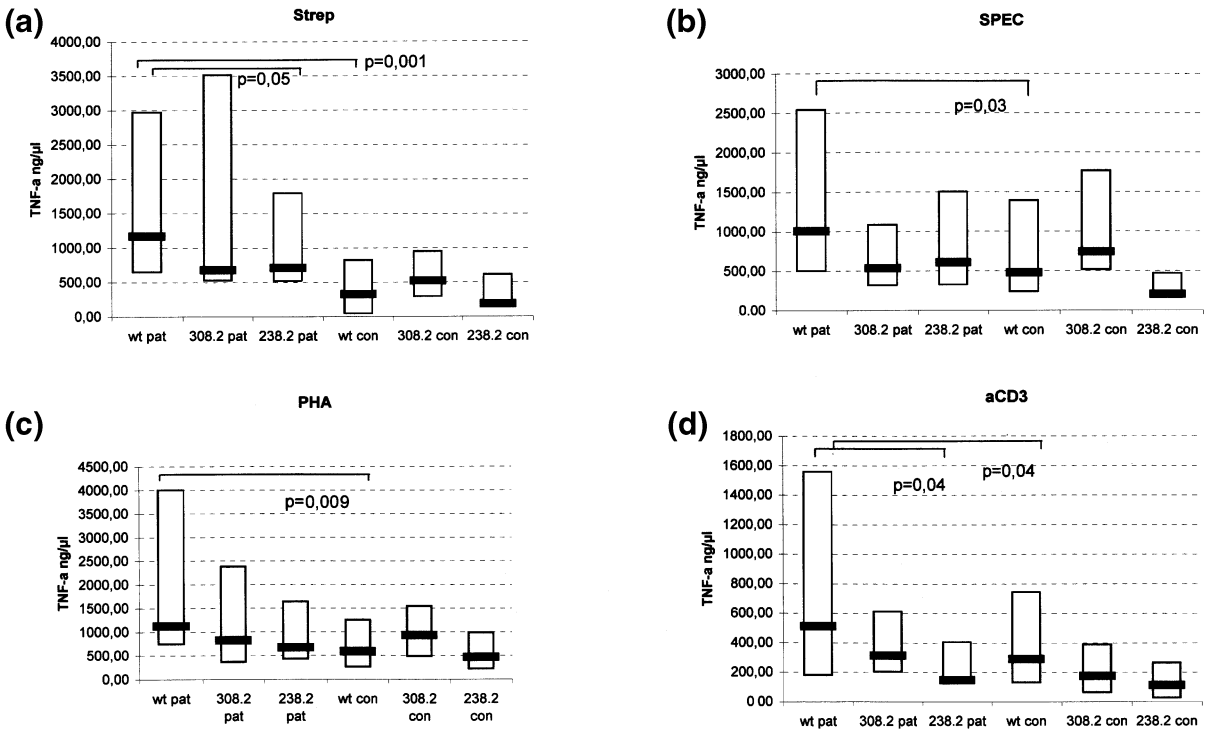

Figure 3. Results of TNF-α-ELISA assays TNF-α production of PBMC after stimulation with *Streptococci* sonicate (Strep, *a*), streptococcal superantigen SPEC (*b*), phytohemagglutinin (*c*), and CD3 monoclonal antibodies (CD3) (*d*). The tested groups are defined as patients (pat) and controls (con) carrying the different promoter alleles (WT, wild-type homozygous; 308.2, TNF308.2/wild-type heterozygous; 238.2, TNF238.2/wild-type heterozygous). The *blocks* represent the quartile 2–4, the *bars* the median of all results; significant differences are marked. Absolute TNF-α production in ng per µl is shown on the *x* axis.

RESULTS

TNF238.2 is increased in psoriasis Distribution of the TNF-α promoter alleles is shown in **Table I**. As expected from earlier studies the TNF238.2 allele was significantly enriched among psoriasis patients ($p < 0.02$). In only two of our probands carrying the TNF238.2 allele the rare –376G→A polymorphism was found.

TNF238A polymorphism decreases promoter activity Results of the luciferase reporter gene assays are shown in **Fig 2**. The TNF308.2 construct had a transcriptional activity comparable to the wild-type construct, whereas the TNF238.2 variant showed a significantly decreased promoter activity after stimulation compared to these two constructs ($p < 0.02$). In the Raji cell line this decreased expression also became obvious in unstimulated cells ($p < 0.04$).

PBMC carrying TNF238.2 produce less TNF-α As shown in **Fig 3**, PBMC from wild-type homozygous psoriasis patients produced more TNF-α than wild-type homozygous controls and other genotypes. Among psoriasis patients, PBMC carrying the TNF238.2 variant produced significantly less TNF-α after stimulation with CD3 ($p < 0.04$) and streptococcal antigens ($p < 0.05$) than wild-type homozygous patients. Controls heterozygous for the TNF238.2 allele had lower TNF-α secretion than either wild-type homozygous or TNF308.2 heterozygous controls, although results did not reach statistical significance because of the small numbers of TNF238.2 heterozygotes. Stimulation of PBMC with the B cell mitogens LPS and PMA did not induce significant differences (data not shown).

DISCUSSION

Genomewide searches have established a strong association of psoriasis with the MHC and in particular with the ancestral haplotype 57.1 (Bhalero and Bowcock, 1998; Jenisch *et al*, 1998, 1999). The exact role of the MHC in the pathogenesis of psoriasis is not yet clear. We now show that the TNF-α promoter allele TNF238.2, which is part of the B57.1 haplotype and found with increased frequency in psoriatic patients, is associated with a significantly decreased transcriptional activity in reporter gene assays. In addition PBMC of TNF238.2 heterozygous individuals show a significantly lower secretion of TNF-α after stimulation with CD3 antibodies and streptococcal sonicates.

TNF-α secretion is predominantly regulated at the transcriptional level (Raabe *et al*, 1998). Interindividual differences in TNF-α secretion are mostly genetically determined (Westendorp *et al*, 1997) and have been associated with HLA-DR haplotypes and polymorphisms in the TNF-α promoter. Particular interest has been focused on the common G→A transition at position –308, but conflicting results have been reported. Increased transcriptional activity of the TNF308.2 allele (Wilson *et al*, 1997) and increased TNF-α production of PBMC carrying this allele have been described (Bouma *et al*, 1996; Louis *et al*, 1998). Findings of other groups including our own, however, suggest that there is no significant effect of this allele on TNF production (Turner *et al*, 1995; Stuber *et al*, 1996; Kroeger *et al*, 1997).

In contrast, the TNF238.2 allele caused a significant decrease in promoter activity compared to the wild-type and the TNF308.2 allele. Fong *et al* (1994) have localized a repressor site (TRS) to a 25 bp stretch between positions –254 and –230 in the promoter. It is tempting to speculate that the –238A exchange could lead to an increased transcriptional repression.

The findings in reporter gene assays are further strengthened by the results of our PBMC studies. TNF-α production of PBMC from patients heterozygous for the TNF238.2 allele showed a decreased TNF-α production after stimulation with different mitogens and antigens. In particular, stimulation with CD3 antibodies, streptococcal sonicates, and streptococcal superantigens led to a significantly decreased TNF-α secretion into supernatants.

This effect was observed in heterozygous psoriasis patients as well as in heterozygous control subjects.

Psoriasis is probably due to a disturbed interaction of T cells and keratinocytes that may be triggered and maintained by streptococcal infections (Valdimarsson *et al*, 1995; Fry, 1998; Bos and de Rie, 1999). TNF-α is secreted mainly by monocytes, macrophages, T cells, and B cells and is found in early psoriatic lesions (Ettehadi *et al*, 1994). It induces the expression of HLA-DR, the production of cell adhesion molecules like intercellular adhesion molecule 1 (Barker *et al*, 1990), and the production of chemoattractants like interleukin-8, which lead to the attraction of inflammatory cells like T lymphocytes and neutrophils (Valdimarsson *et al*, 1995). The pathogenetic consequences of our findings remain to be defined. Decreased TNF-α secretion could characterize psoriasis patients with a particular disease course or be related to an impaired clearance of skin infections with candida or streptococci, and thus predispose individuals to the development of the disease.

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REFERENCES

Allen MH, Veal C, Faasesen A, Powis SH, Vaughan RW, Trembath RC, Barker JNW: A non-HLA gene within the MHC in psoriasis. *Lancet* 353:1589–1590, 1999

Arias AI, Giles B, Eiermann TH, Sterry W, Pandey JP: Tumor necrosis factor-alpha gene polymorphism in psoriasis. *Exp Clin Immunogenet* 14:118–122, 1997

Barker JN, Sarma V, Mitra RS, Dixit VM, Nickoloff BJ: Marked synergism between tumor necrosis factor-alpha and interferon-gamma in regulation of keratinocyte-derived adhesion molecules and chemotactic factors. *J Clin Invest* 85:605–608, 1990

Bhalero J, Bowcock AM: The genetics of psoriasis: a complex disorder of the skin and immune system. *Hum Mol Genet* 7:1537–1545, 1998

Bos JD, de Rie MA: The pathogenesis of psoriasis: immunological facts and speculations. *Immunol Today* 20:40–46, 1999

Bouma G, Crusius JBA, Oudkerk Pool M, *et al*: Secretion of TNF-alpha and LTα in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. *Scand J Immunol* 43:456–463, 1996

D'Alfonso S, Richiardi PM: A polymorphic variation in a putative regulation box of the TNFA promoter region. *Immunogenetics* 39:150–154, 1994

Elder JT, Henseler T, Christophers E, Voorhees JJ, Nair RP: The genetics of psoriasis. *Arch Dermatol* 130:216–224, 1994

Ettehadi P, Greaves MW, Wallach D, Aderka K, Camp RD: Elevated tumor necrosis factor-alpha biological activity in psoriasis lesions. *Clin Exp Immunol* 96:145–151, 1994

Fong CW, Siddiqui AH, Mark DF: Identification and characterisation of a novel repressor site in the human tumor necrosis factor alpha gene. *Nucl Acid Res* 22:1108–1114, 1994

Fry L: Psoriasis. *Br J Dermatol* 119:445–461, 1998

Gallagher G, Eskdale J, Oh HH, Richards SD, Campbell DA, Field M: Polymorphisms in the TNF gene cluster and MHC serotypes in the West of Scotland. *Immunogenetics* 45:188–194, 1997

Gladman DD: Natural history of psoriatic disease. *Baillere's Clin Rheum* 8:379–394, 1994

Hamann A, Mantzoros C, Vidal-Puig A, Flier JS: Genetic variability in the TNF-α promoter is not associated with type II diabetes mellitus (NIDDM). *Biochem Biophys Res Commun* 211:833–839, 1995

Hoehler T, Kruger A, Schneider PM, *et al*: A TNF-alpha-promoter polymorphism is associated with juvenile onset psoriasis and psoriatic arthritis. *J Invest Dermatol* 109:562–556, 1997

Jenisch S, Henseler T, Nair RP, *et al*: Linkage analysis of human leukocyte antigen (HLA) markers in familial psoriasis: strong disequilibrium effects provide evidence for a major determinant in the HLA-B/-C region. *Am J Hum Genet* 63:191–199, 1998

Jenisch S, Westphal E, Nair RP, *et al*: Linkage disequilibrium analysis of familial psoriasis: identification of multiple disease-associated MHC haplotypes. *Tissue Antigens* 53:135–146, 1999

Jongeneel CV, Briant L, Udalova IA, Sevin A, Nedospasov SA, Cambon-Thomsen A: Extensive genetic polymorphism in the human tumor necrosis factor region and relation to extended HLA haplotypes. *Proc Natl Sci USA* 88:9717–9721, 1991

Knight JC, Udalova I, Hill AVS, Greenwood BM, Peshu N, Marsh K, Kwiatkowski D: A polymorphism that affects OCT-1 binding to the TNF promoter region is associated with severe malaria. *Nature Genet* 22:145–150, 1999

Kroeger KM, Carville KS, Abraham LJ: The –308 tumor necrosis factor alpha promoter polymorphism effects transcription. *Mol Immunol* 34:391–399, 1997

Louis E, Franchimont D, Piron A, *et al*: TNF gene polymorphism influences TNF-α-production in LPS-stimulated whole blood cell cultures in healthy humans. *Clin Exp Immunol* 113:401–406, 1998

Nedospasov SA, Udalova IA, Kuprash DY, Tretskya RL: DNA sequence polymorphism at the human tumor necrosis factor locus. *J Immunol* 147:1053–1059, 1991

Pociot F, D'Alfonso S, Compasso S, Scorza R, Richiardi PM: Functional analysis of a new polymorphism in the human TNF-α gene promoter. *Scand J Immunol* 42:501–504, 1995

Raabe T, Bukrinsky M, Currie RA: Relative contribution of transcription and translation of the induction of tumor necrosis factos alpha by lipopolysaccharide. *J Biol Chem* 273:974–980, 1998

Skoog T, van't Hooft FM, Kallin B, *et al*: A common functional polymorphism (C to A substitution –863) in the promoter region of the TNF-α gene associated with reduced circulating levels of TNF-α. *Hum Mol Genet* 8:1443–1449, 1999

Stuber F, Udalova IA, Book M, *et al*: –308 TNF polymorphism is not associated with survival in severe sepsis and is unrelated to LPS inducibility of the huamn TNF promoter. *J Inflammation* 46:42–50, 1996

Turner DM, Grant SC, Lamb WR, Brenchley PE, Dyer PA, Sinnott PJ, Hutchinson IV: A genetic marker of high TNF-alpha production in heart transplant recipients. *Transplantation* 60:1113–1117, 1995

Valdimarsson H, Baker BS, Jonsdottir I, Powles A, Fry L: Psoriasis: a T-cell mediated autoimmune disease induced by streptococcal superantigens? *Immunol Today* 16:145–114, 1995

Westendorp RG, Langermans JAM, Huizinga TWJ, Elouali AH, Verweij CL, Boomsma DI, Vandenbrouke JP: Genetic influence on cytokine production and fatal meningococcal disease. *Lancet* 349:170–173, 1997

Wilson AG, Di Giovine FS, Blakemore AIF, Duff GW: Single base change in the human tumour necrosis factor alpha gene detectable by Nco I restriction of the PCR product. *Hum Mol Genet* 1:353, 1992

Wilson AG, Symons JA, McDowell TL, Di Giovine FS, Duff GW: Effects of a TNF-alpha promoter base transition on transcriptional activity. *Proc Natl Acad Sci USA* 94:3195–3199, 1997